pressures and produce non-linear scattering with moderate acoustic driving pressures. At moderate acoustic driving pressures, microbubbles exhibit pressure peaks at the compressional phases of the source thereby providing both harmonic and subharmonic energy greater than the surrounding medium. At very high acoustic driving pressures microbubbles cavitate or destruct as a result of fragmentation and deflation and thus create an associated acoustic emission signal. The absolute values for low, moderate and high acoustic driving pressures are not well defined and depend upon not only the acoustic parameters of the ultrasonic source but also the constituent physical properties of the microbubbles themselves, as well as the fluid surrounding them.

A significant problem with the use of microbubble contrast agents result from the machinery associated with the imaging process. Typical medical diagnostic ultrasound imaging machinery produces acoustic pressures that can range from 0.5 to 3 mega pascals (MPa). This acoustic pressure range can destroy some microbubble contrast agents during the imaging process, thus reducing the efficacy of the contrast agent and also reducing the effective imaging time (half-life) of the contrast agent.

Albunex® (from Molecular Biosystems, of San Diego, CA), the first commercially available ultrasound contrast agent, is a suspension of air-filled albumin microspheres produced by sonication of a heated solution of 5% human albumin. The major drawbacks associated with use of Albunex® as a contrast agent for ultrasound are its short plasma half-life and its acoustic instability relative to pressure changes. The plasma half-life of radiolabeled Albunex® microbubbles after intravenous injection is less than one minute. In addition, backscatter intensity falls as pressure rises, an effect that has been demonstrated *in*

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vivo as a systolic fall in videointensity following intravenous injection. Moreover, albumin microbubbles cannot by used with other modalities such as magnetic resonance imaging or computed tomography because the microbubbles do not have the functional characteristics required for such modalities.

With the development of medical ultrasonic contrast agents, the theoretical behavior of encapsulated microbubbles has generated substantial interest. Ye found that at frequencies below or slightly higher than the resonance, acoustic scattering by Albunex® bubbles is nearly omni-directional and bears similarities to that by usual air bubbles. (Ye, "On Sound Scattering and Attenuation of Albunex® Bubbles," *J. Acoust. Soc. Am.*, 100(4) 2011-28, (1995)). The Ye reference also reveals that the scattering by Albunex® bubbles can be highly anisotropic when the frequency is above resonance. Work by de Jong showed large differences in non-linear behavior between ideal and Albunex® microspheres due to the additional restoring force and friction inside the shell that surrounds the Albunex® microsphere. (de Jong et al, "Higher Harmonics of Vibrating Gas-Filled Microspheres, Part One: Simulations," *Ultrasonics*, 32(6) 447-453 (1994)).

Prior efforts to address the need for an increase in the plasma half-life of medical ultrasonic contrast agents have focused on: (1) strengthening the structure of the encapsulating shell, (2) employing different substances for the encapsulating shell, or (3) chemical modification of the microsphere surface, for example, by pegylation. For example, the use of galactose with human serum albumin microspheres appears to strengthen the shell, thereby increasing the half-life to 3 to 6 minutes. (Goldberg, "Ultrasound Contrast Agents," *Clin. Diag. Ultrasound*, 28:35-45 (1993)). Kimura et al. utilized small unilamellar vesicle

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("SUV"), large unilamellar vesicle ("LUV") and multilamellar vesicle ("MLV") as echogenic liposomes. (Kimura et al., "Preparation and Characterization of Echogenic Liposome as an Ultrasound Contrast Agent," *Chem. Pharm. Bull.*, 46(10) 1493-96 (1998)). The acoustic reflectivity obtained with the echogenic MLV was larger than that of the gas bubbles enclosed within a surfactant mixture. A half-lifetime of 39 minutes was observed for the MLV prepared from egg-yolk phosphatidylcholine liposomes. The duration of reflectivity was prolonged drastically to a half-lifetime of 866 minutes by incorporating cholesterol into the MLV, although, significantly, the echogenicity was decreased by such incorporation. Although there have been a number of important steps at lengthening the effective imaging half-life of injectable ultrasonic contrast agents using liposomes, there has been an overall reduction in the echogenicity of these agents.

Thus, although there are a number of ultrasonic contrast agents now available commercially, and despite significant research directed to many of these agents, limitations still exist with these agents. Furthermore, few ultrasonic contrast agents can be used with other imaging modalities.

Magnetic Resonance

Another imaging technique is magnetic resonance ("MR") imaging. This modality relies on detecting the emission of electromagnetic radiation by certain atomic nuclei in the body upon application of pulsed radio frequency signals in the presence of a magnetic field. The resulting magnetic echoes produced when the signal is terminated ultimately are translated into an image.

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